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Short Communication

Action Spectrum of Cytochrome *f* Photooxidation in Greening Bean Leaves

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Photooxidation of cytochrome *f* can be demonstrated in an etiolated bean leaf after 2 to 3 hr of illumination (3, 4). Photosystem II activity as measured by the ability of 675 nm of light to hold cytochrome *f* mainly in the reduced state is observed shortly afterwards. These events coincide with the end of the lag phase of chlorophyll synthesis, but new chlorophyll synthesis does not appear to be necessary for the photooxidation of cytochrome *f*. Etiolated leaves illuminated for 3 min and then returned to darkness for 2 to 3 hr show similar light-driven redox reactions of cytochrome *f*, although little new chlorophyll is synthesized under these conditions (4). If such leaves are treated with DCMU to inhibit the photoreduction of cytochrome *f*, it is found that light of wavelength 688 nm is slightly more effective than 667 nm or 675 nm of light in photooxidizing cytochrome *f*, even though the *in vivo* absorption maximum of the leaf is at 672 nm (4). In the present study, we have determined the action spectrum for the photooxidation of cytochrome *f* and compared it with the absorption spectrum of the leaf. The action spectrum shows a maximum at 682 nm, compared with 672 nm for the absorption spectrum.

MATERIALS AND METHODS

Dwarf French beans (*Phaseolus vulgaris* L. var. Brown Beauty) were grown in darkness at 25 ± 1 C for 10 to 15 days. Trays of plants were exposed to fluorescent white light (400 ft-c) for 3 min and then returned to darkness for 6 hr. At this time large primary leaves were excised, pricked with a needle, and placed in 10 μM DCMU for 15 min and then re-exposed to continuous white light. Light-driven absorbance changes due to cytochrome *f* were measured in an Aminco-Chance dual wavelength spectophotometer equipped with a cross illumination attachment. The measuring wavelength was 554 nm, and the reference wavelength was 570 nm. Actinic light was obtained from an "Eimac" xenon arc source (150 w) and appropriate wavelengths selected by means of a Corning 2–60 glass filter and a Bausch and Lomb 500 mm monochromator to give a half-band width of 5 nm (1.5-mm slits). All experiments were completed within 1 hr of exposing the leaf to the light for the second time. Whole leaf absorption spectra were measured in a Cary Model 14R spectrophotometer equipped with a scattered transmission accessory, as described previously (6).

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RESULTS AND DISCUSSION

Our previous studies (4) showed that the rate of cytochrome *f* oxidation was proportional to light intensity over a 10-fold range of intensity (1.5×10^3 to 15×10^3 erg cm^{-2} sec^{-1}). In the present work, the intensity incident on the leaf surface (3.9×10^3 ergs cm^{-2} sec^{-1}) was within this proportional range. The action spectrum for the photooxidation of cytochrome *f* together with the absorption spectrum of the leaf *in vivo* is shown in Figure 1. There is a clear maximum in the action spectrum at 682 nm with a possible secondary peak at 678 nm and a slight shoulder at 670 nm. In contrast, the absorption spectrum of the leaf shows a maximum at 672 nm with only the slightest, barely detectable shoulder in the region of 682 nm. It is apparent, therefore, that a large proportion of the chlorophyll of the leaf in the early stages of greening is inactive in the photooxidation of cytochrome *f*. This inactive chlorophyll might be that associated with photosystem II, although the rate of cytochrome *f* oxidation at 703 nm was increased 2- to 6-fold by the addition of DCMU (4), suggesting that there is considerable absorption of photosystem II at long wavelengths under our conditions. We attempted to obtain an action spectrum for photosystem II by measuring the photoreduction in the intact leaf of the electron acceptors, 2,6-dichlorophenolindophenol, ferricyanide, and tetranitro blue tetrazolium, but we were unsuccessful. This may be due to the failure of these Hill oxidants to pentrate the leaf.

The peak in the action spectrum at 682 nm is close to that observed by Joliot *et al.* (5) for photosystem I in spinach chloroplasts. This agreement is probably coincidental, since the chlorophyll content of a spinach leaf per unit area is 30- to 40-fold higher than the protochlorophyllide content of an etiolated bean leaf. The chlorophyll/cytochrome *f* molar ratio of a spinach leaf is about 400, but the primary bean leaves used in this study had a chlorophyll/cytochrome *f* ratio of approximately five (4). In most of the leaves we have used more than half the total cytochrome *f* can be photooxidized. Since a large proportion of the chlorophyll is inactive or associated with photosystem II, the greening bean leaves must contain a high proportion of photosystem I units in which one or two chlorophyll molecules are associated with one cytochrome *f* molecule. It appears, therefore, that the reaction center chlorophyll of photosystem I in the early stages of greening has an absorption maximum close to 682 nm. P-700 is considered to be the reaction center chlorophyll of photosystem I in mature leaves (7, 8), but in recent studies with plastids from greening pea leaves (2), a light-induced absorbancy change at 700 nm was not observed during the early stages of greening.

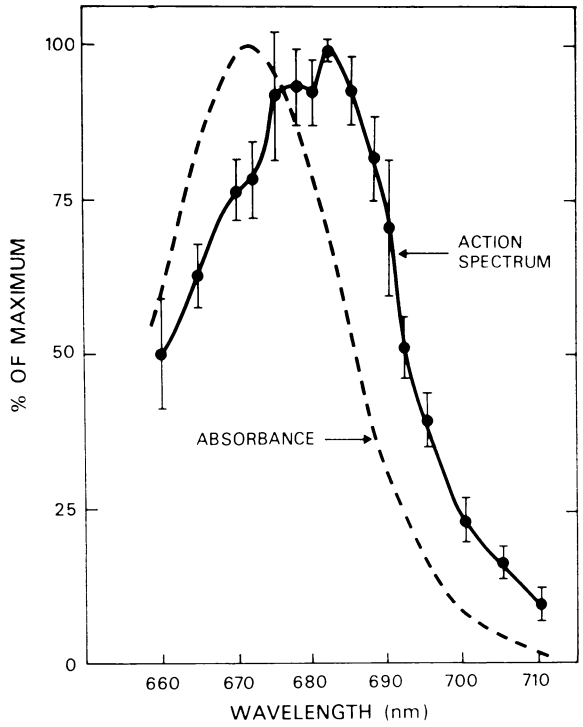

FIG. 1. Action spectrum for the photooxidation of cytochrome *f* in etiolated bean leaves. Seedlings were exposed to white light for 3 min and returned to darkness for 6 hr. Primary leaves were treated with 10 μM DCMU for 15 min and re-exposed to light. ●—●, rate of cytochrome oxidation. -----, *in vivo* absorption spectrum of a leaf. The points on the action spectrum are average measurements obtained from two leaves. A total of seven runs from high to low actinic wavelengths was made, but not all wavelengths were included in each run. At least six measurements were made for each point in the range 690 to 675 nm. The vertical bars represent the standard deviations of values obtained. The incident light intensity was 3.9×10^3 ergs cm^{-2} sec^{-1} and the maximum rate of cytochrome *f* oxidation was 0.4 nmole cm^{-2} min^{-1}. The absorbance of the leaf at 672 nm was 0.07.

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